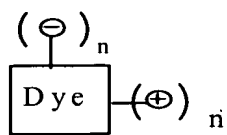
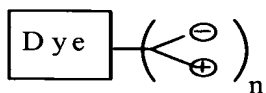




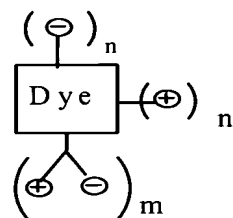
1/19



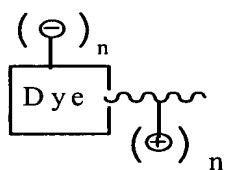
A



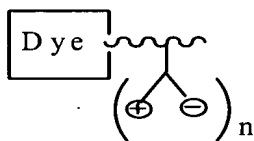
B



C

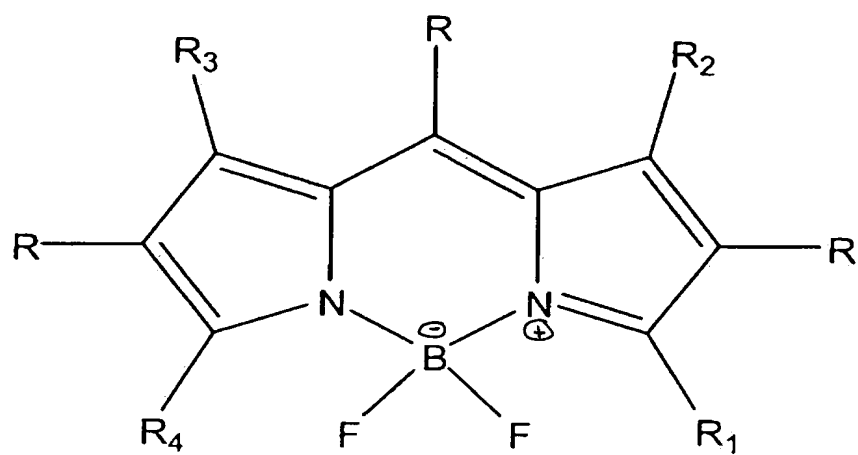


D



E

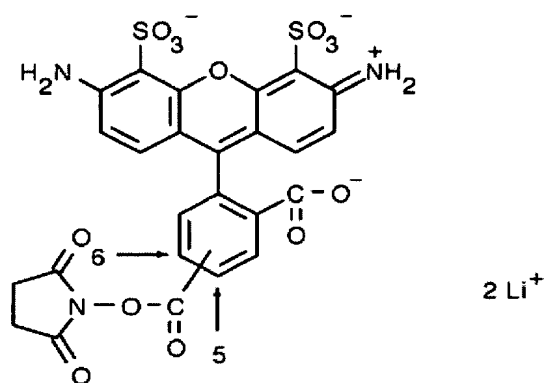
FIGURE 1



BODIPY fluorophore, 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene

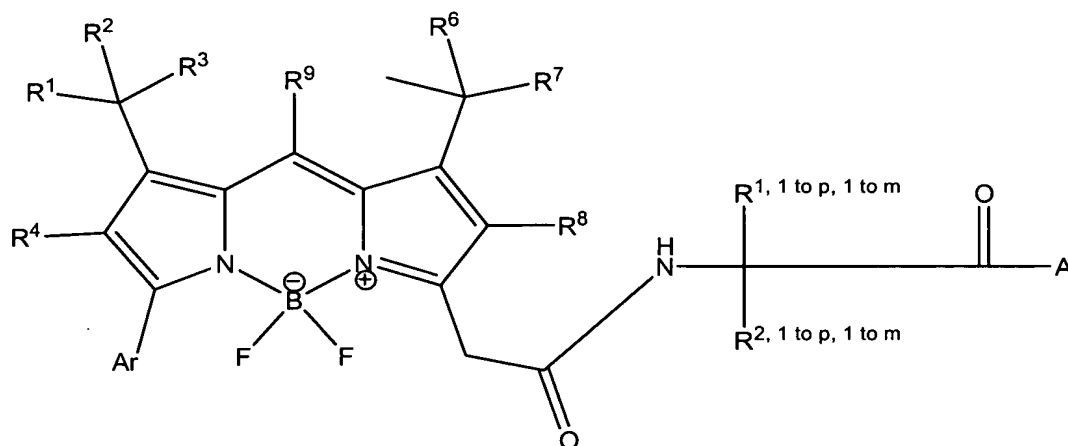
FIGURE 2

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Alexa Fluor ® 488 carboxylic acid, succinimidyl ester dye structure

FIGURE 3



General structure of an optical labeling molecule comprising a BODIPY dye moiety

A = Ester activator, NHCH₂CH₂SH, or other linker

R₁ to R₉ = to be defined

R₁, 1 to p, 1 to m and R₂, 1 to p, 1 to m = to be defined

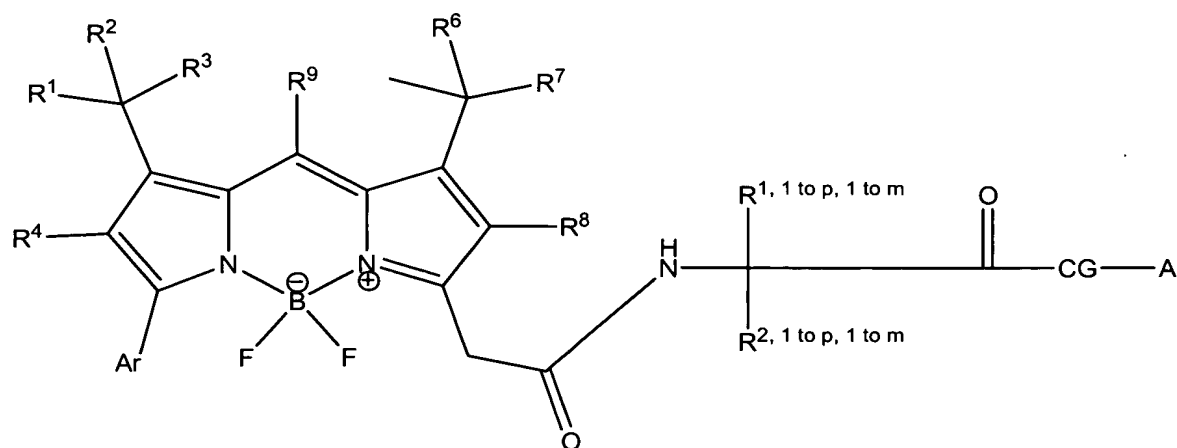
The R groups must be combined to have an equal number of non-titratable positive and negative groups to produce zwitterionic pairs

Ar = Aryl

r, n, m, p, q = 0, 1, 2, 3...

For each value of p, there are p values of m. These p values can be equal or different

FIGURE 4A



General structure of an optical labeling molecule comprising a BODIPY dye moiety

A = Ester activator, NHCH₂CH₂SH, or other linker

CG = Cleavable group

R¹ to R⁹ = to be defined

R¹, 1 to p, 1 to m and R², 1 to p, 1 to m = to be defined

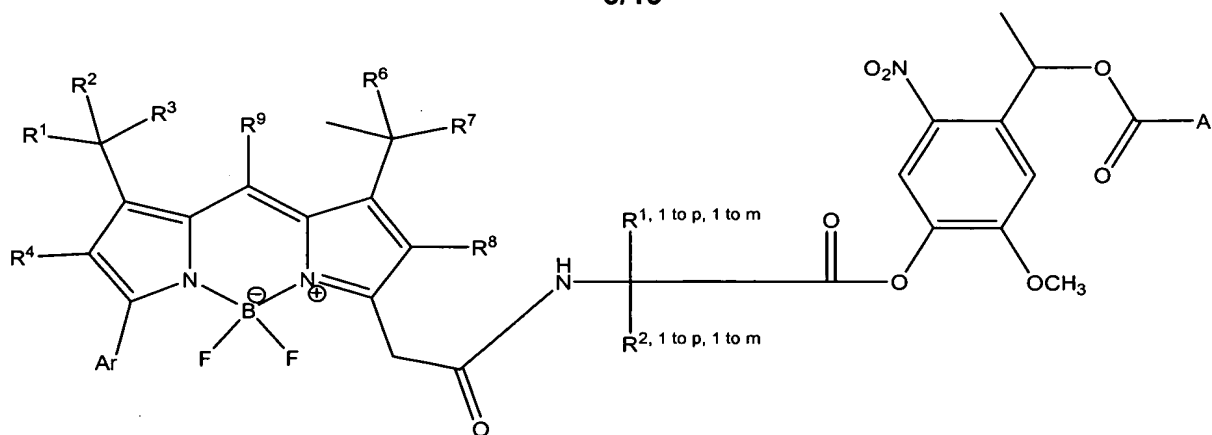
The R groups must be combined to have an equal number of non-titratable positive and negative groups to produce zwitterionic pairs

Ar = Aryl

r, n, m, p, q = 0, 1, 2, 3...

For each value of p, there are p values of m. These p values can be equal or different

FIGURE 4B



General structure of an optical labeling molecule comprising a BODIPY dye moiety with a p-nitro anisole group

A = Ester activator, NHCH₂CH₂SH, or other linker

R₁ to R₉ = to be defined

R₁, 1 to p, 1 to m and R₂, 1 to p, 1 to m = to be defined

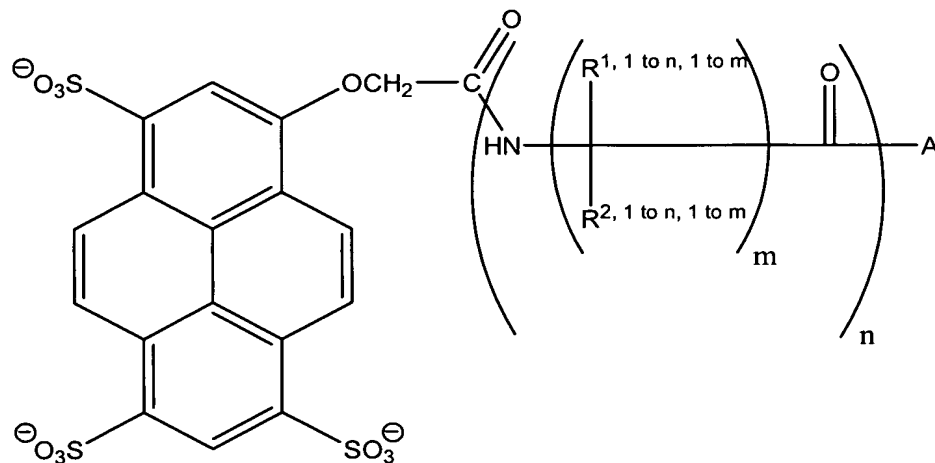
The R groups must be combined to have an equal number of non-titratable positive and negative groups to produce zwitterionic pairs

Ar = Aryl

r, n, m, p, q = 0, 1, 2, 3...

For each value of p, there are p values of m. These p values can be equal or different

FIGURE 5



General structure of an optical labeling molecule comprising a Cascade Blue dye moiety

$n, m = 1, 2, 3, \dots$

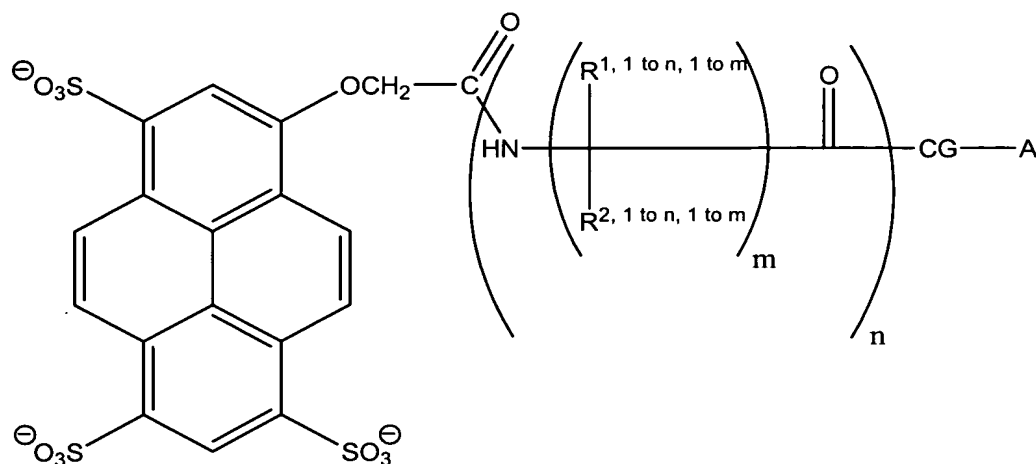
$\text{R}^1, 1 \text{ to } n, 1 \text{ to } m$ and $\text{R}^2, 1 \text{ to } n, 1 \text{ to } m =$ to be defined

Three non-titratable cationic groups must be included in the R groups

A = nucleophilic attack activator

For each value of n , there are n values of m . These n values can be equal or different

FIGURE 6A



General structure of an optical labeling molecule comprising a Cascade Blue dye moiety

$n, m = 1, 2, 3, \dots$

$\text{R}^1, 1 \text{ to } n, 1 \text{ to } m$ and $\text{R}^2, 1 \text{ to } n, 1 \text{ to } m =$ to be defined

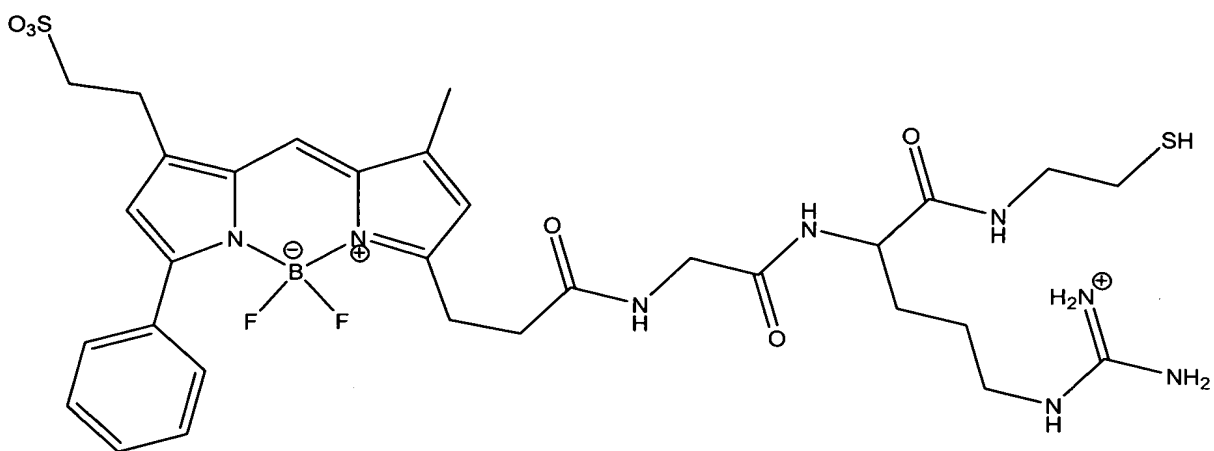
Three non-titratable cationic groups must be included in the R groups

CG = cleavable group

A = nucleophilic attack activator

For each value of p , there are p values of m . These p values can be equal or different

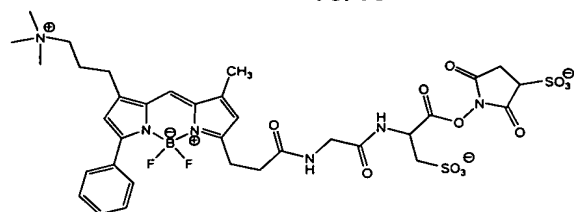
FIGURE 6B



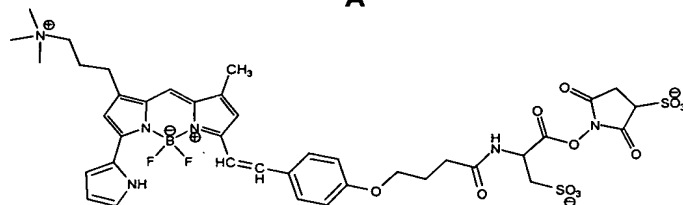
General structure of an optical labeling molecule that can be used to label phosphorylation sites on proteins after beta-elimination of the phosphates from serine or threonine residues.

FIGURE 7

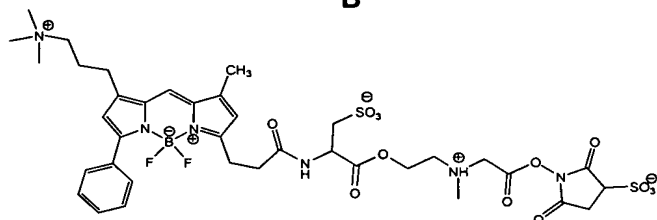
10/19



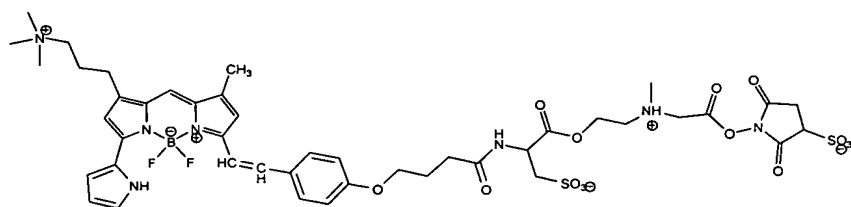
A



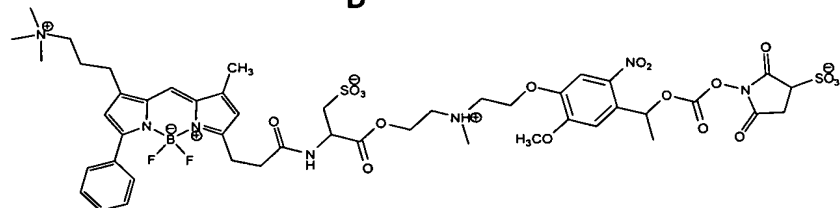
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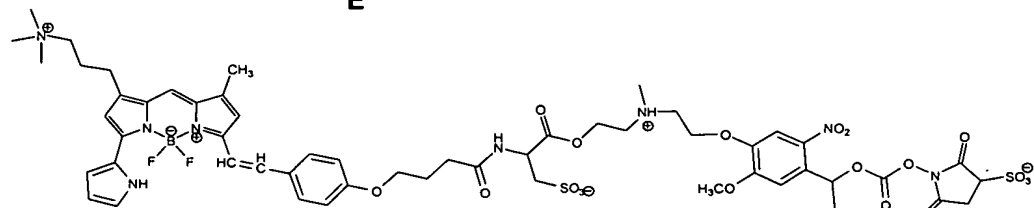
C



D



E



F

FIGURE 8A

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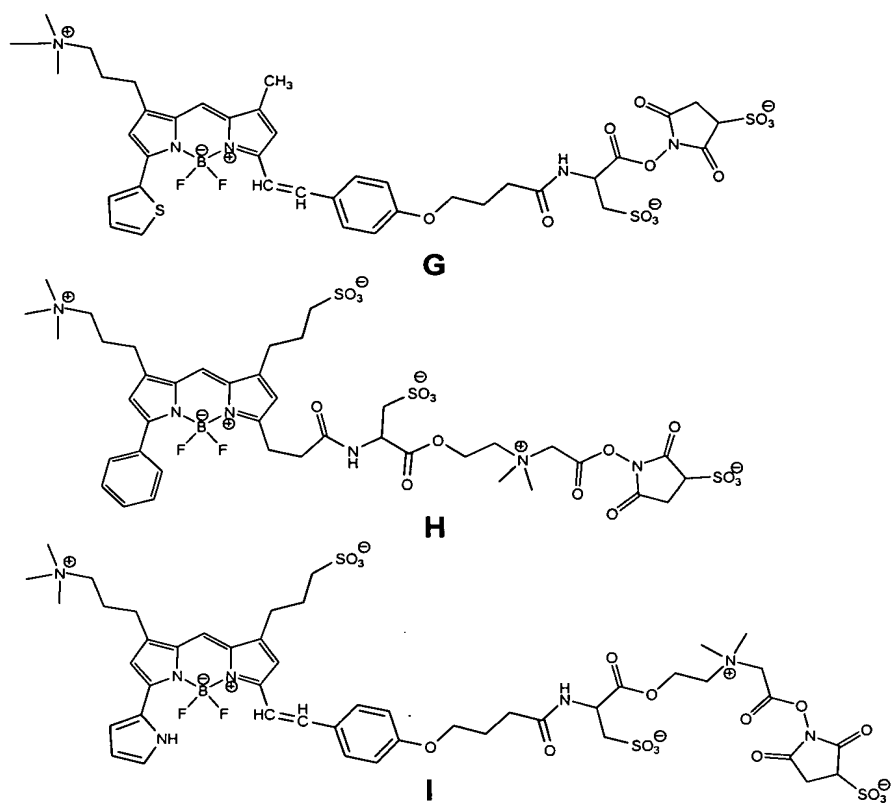
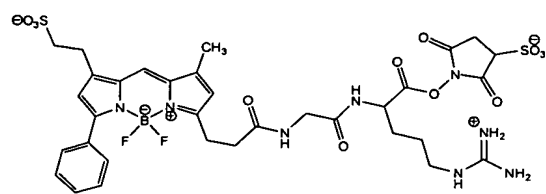
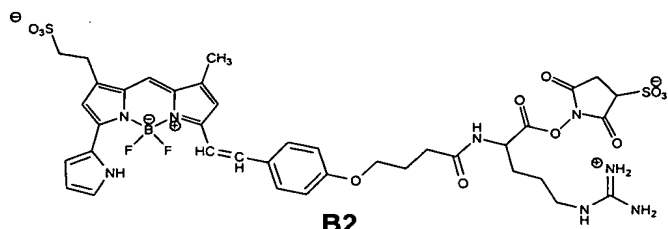


FIGURE 8B

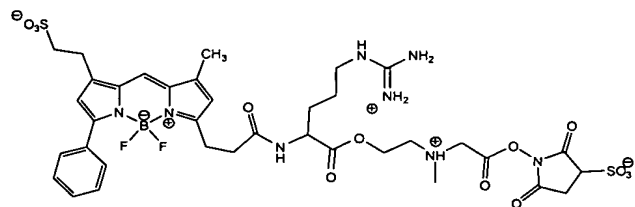
12/19



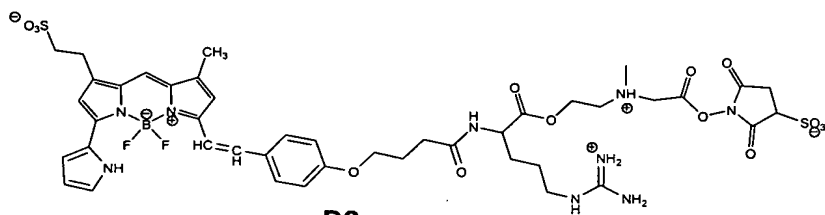
A2



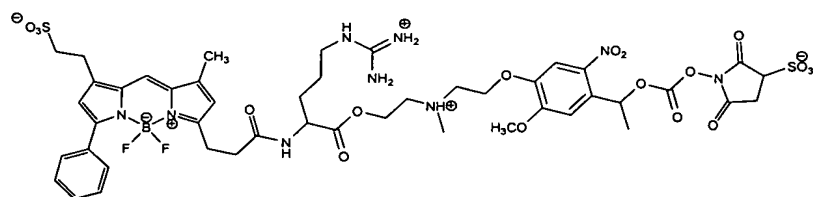
B2



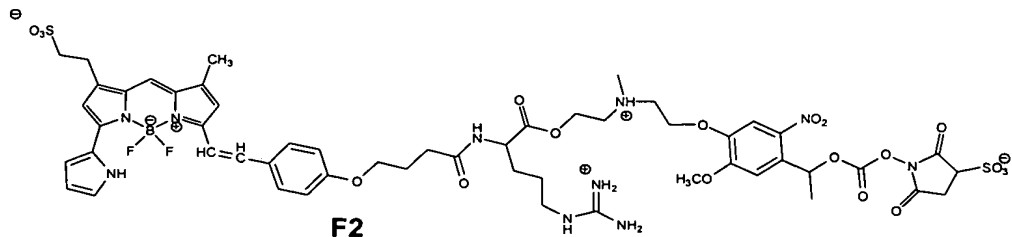
C2



D2



E2



F2

FIGURE 9A

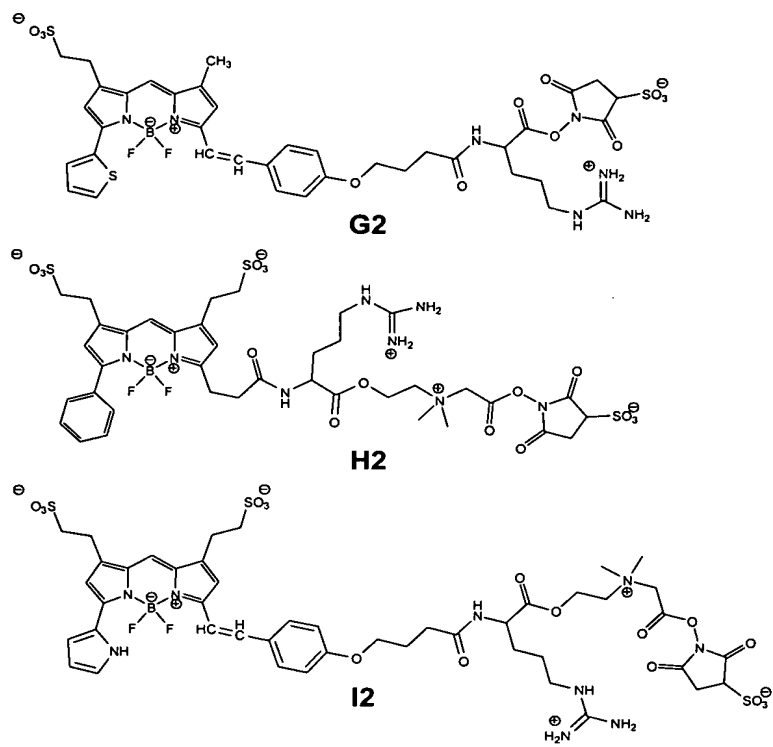


FIGURE 9B

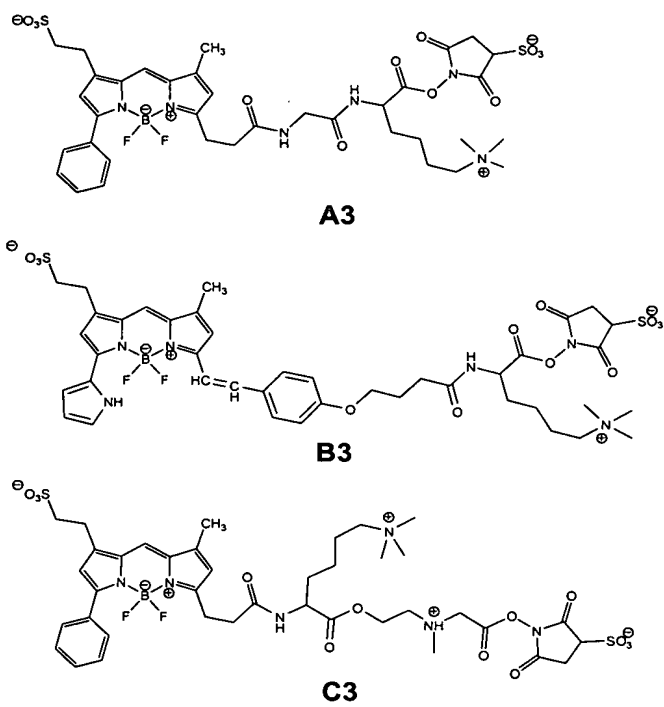
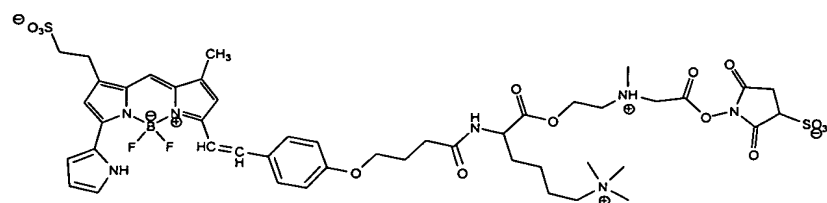
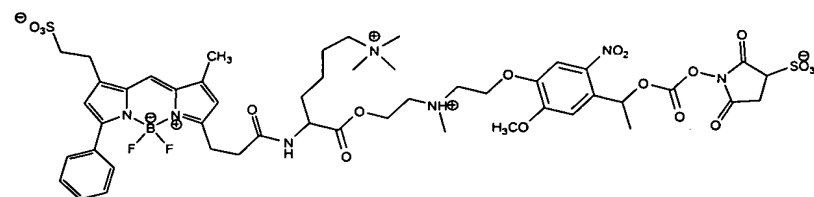
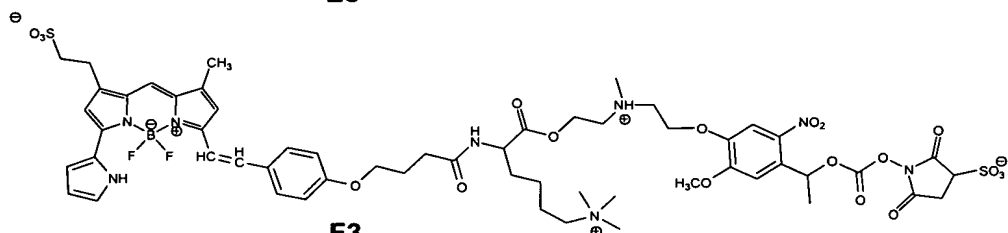
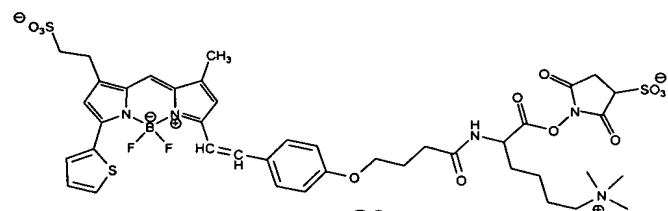
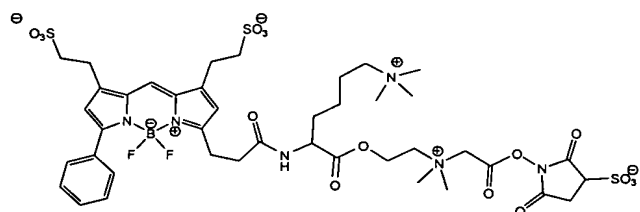
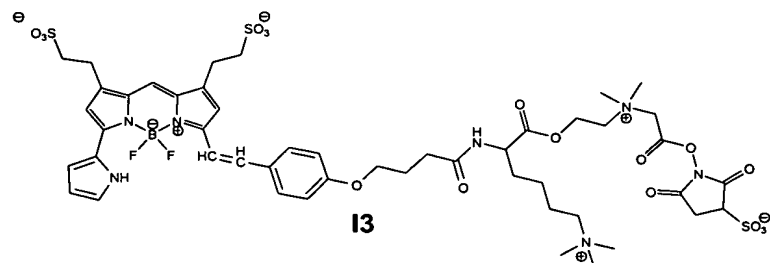
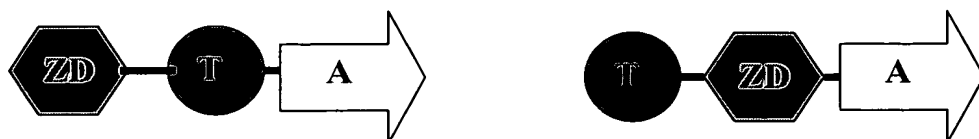


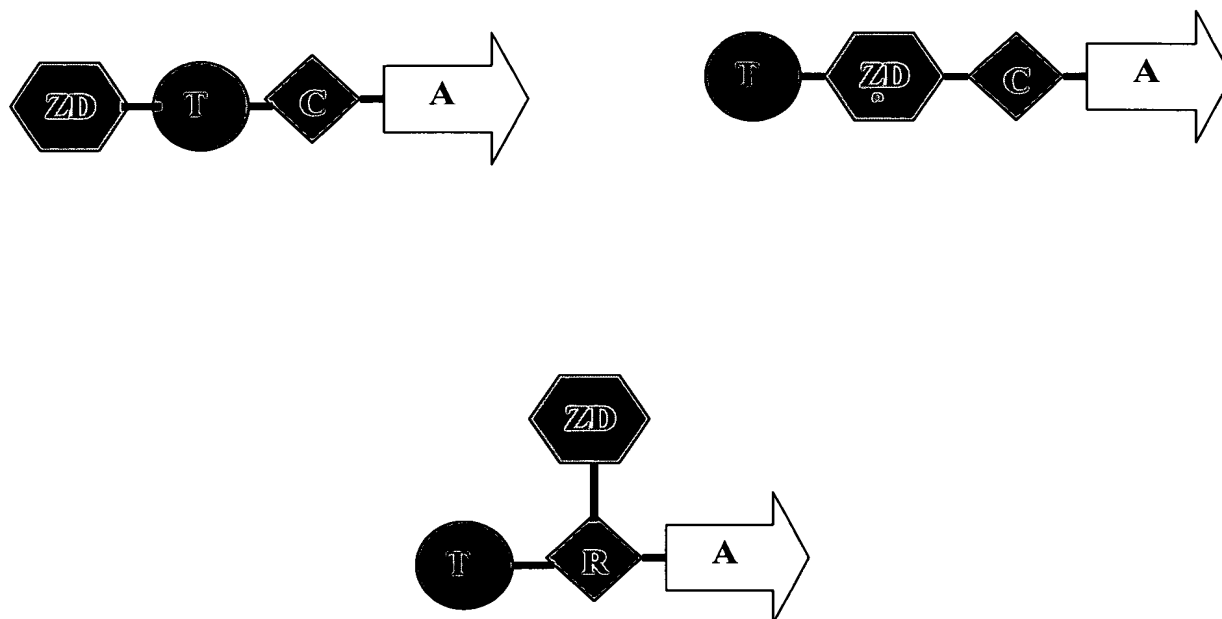
FIGURE 10A

**D3****E3****F3****G3****H3****I3****FIGURE 10B**



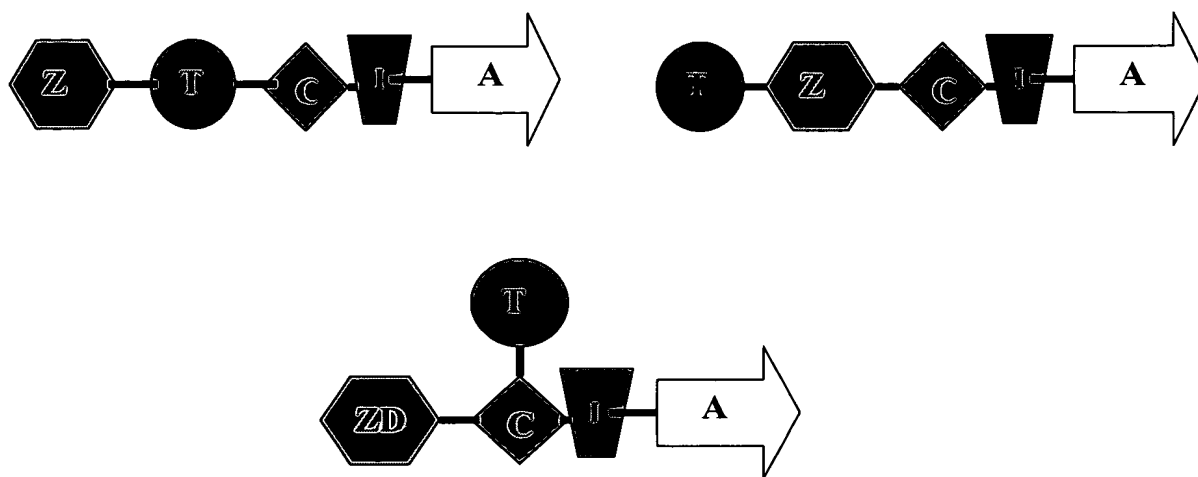
General structure of an optical labeling molecule wherein ZD is the zwitterionic dye moiety, T is the titratable group moiety, and A is the functional linker.

FIGURE 11



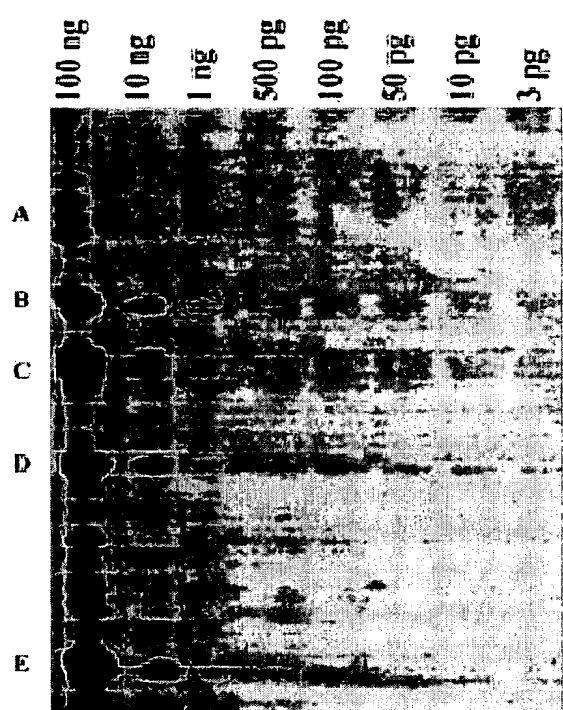
General structures of an optical labeling molecule wherein ZD is the zwitterionic dye moiety, T is the titratable group moiety, C is the cleavable moiety and A is the functional linker.

FIGURE 12



General structures of an optical labeling molecule wherein ZD is the zwitterionic dye moiety, T is the titratable group moiety, C is the cleavable moiety, I is the stable isotope moiety and A is the functional linker.

FIGURE 13



Gel showing the detection sensitivity obtained by prelabeling a set of standard proteins in SDS using a BODIPY dye from Molecular Probes

FIGURE 14